

## RETROSPECTIVE STUDIES ON BOVINE CALVES AFFECTED WITH CONGENITAL HEART DISEASE

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### ABSTRACT

Congenital heart disease (CHD) was reported in 4 necropsied calves referred to Mansoura veterinary teaching hospital during the period 2014-2016. The Four necropsied calves suffered from patent foramen ovale (PFO) (n=1), high ventricular septal defect (HVSD) (n=2) and persistent ductus arteriosus (PDA) (n=1). VSD was accompanied by valvular hematoma in 1 calf, hydropericardium, right ventricular hypertrophy and dextroposition of the aorta in another calf. *Pasteurella multocida* (*P. multocida*) was isolated from one lung lesion associated with VSD. Histopathological examination of different tissues from necropsied CHD affected calves was made. We concluded that extramedullary hematopoiesis in liver and lymphoid organs besides chronic interstitial nephritis were constant findings associated with CHD in calves.

**Key words:** Congenital heart disease, calves, Egypt

### INTRODUCTION

Congenital heart disease (CHD) in calves was uncommon being observed in only 0.17% of 50742 bovine hearts inspected in a slaughterhouse study (Van Nie, 1966). In a retrospective study of 2293 calves with congenital anomalies, cardiac malformations occurred in 2.7% of the cases (Leipold *et al.*, 1972). Ventricular septal defect (VSD) is the most common cardiovascular abnormality in bovine neonates (Fischer and Pirie 1965; Van Nie, 1966; Gopal *et al.*, 1986; West, 1988; Ohwada *et al.*, 2000). Heart defects were diagnosed in 11 of 36 and 7 of 10 calves in 2 separate studies (Leipold *et al.*, 1972; West, 1988). VSD in cattle has also been identified in combination with multiple cardiac defects, such as dextroposition of the aorta (Belling 1961 and 1962; Van Nie, 1966, Sandusky and Smith 1981; West, 1988; Penrith *et al.*, 1994; Gavaghan *et al.*, 2001), persistent ductus arteriosus (PDA) (Cordy and Ribelin, 1950; Sandusky and Smith 1981; West, 1988), patent foramen ovale (PFO) (West, 1988; Penrith *et al.*, 1994; Schwarzwald *et al.*, 2003), PDA (Belling 1961 and 1962), transposition of the great vessels (Sandusky and Smith 1981; West, 1988) and anomaly of the pulmonary (Belling 1961; Schwarzwald *et al.*, 2003) or tricuspid valves (West, 1988). PDA is a persistent patency of a vessel (normally present in the fetus) that connects the

pulmonary arterial system to the aorta. Closure of the ductus arteriosus when breathing commences and placental blood circulation is removed in response to decline pulmonary vascular resistance and increased systemic vascular resistance (Gournay, 2011). The cause of congenital cardiac abnormalities was still unknown. However, it was supposed that CHD may result from injury during development or from single recessive genes or polygenic sets that have lesion-specific effects on cardiac development (Smith, 2009; Jafari Dehkordi *et al.*, 2010). PDA may be accidentally found in calves as previously reported during an experiment on an implantable ventricular assist device in Korean calf (Lee and Kim, 2004). In this report we described histopathology of tissues in CHD affected calves and correlated our findings to bacteriological and immunohistochemical parameters.

### MATERIALS AND METHODS

Seven calves referred to Mansoura veterinary teaching hospital, 2014-2016 were necropsied due to severe weakness, respiratory distress or abnormal cardiac sounds. Diagnosis of those anomalies was based on history, clinical examination that was dependent upon visual inspection, auscultation and gross postmortem examination. After internal examination was completed in necropsied calves, tissue samples were collected from different organs and fixed in 10% neutral formalin. Paraffin sections were made and 5 µm sections were cut, mounted on glass microscope slides and stained with hematoxylin and eosin (H&E). Specimens from infectious lung lesion were sent to bacteriological Lab for isolation.

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In addition, paraffin tissue sections from such lung lesion were placed on positively charged slides (coated slides), deparaffinized and dehydrated. After rinsing in PBS, 3% hydrogen peroxide was applied for 5 minutes to block the endogenous peroxidase. The sections were immunostained using rabbit monoclonal anti-*P. multocida* antibody with dilution of 1:4000 in PBS. After washing with PBS, slides were incubated at 37°C for 45 minutes with Envision and anti-rabbit –horse radish peroxidase labeled polymer kit, DAKO (Carpenteria, CA). The IHC reaction was developed using 3, 3-diaminobenzidine (DAB) (from DAKO Carpenteria, CA) for 5-10 minutes. Sections were counterstained with Mayer's Hematoxylin. Test slides incubated with PBS were used as negative control sections. All slides were examined by light microscopy and the intensity of immunostaining was recorded (Awadin *et al.*, 2012).

## RESULTS

### I- Case history and clinical findings:

A diagnosis of CHD was done in 4 calves based on a history of weakness, ill thrift, poor growth and respiratory disease that failed to respond to appropriate therapy. On physical examination, heart murmur was auscultated on the left and right cardiac area. Tachycardia and increase respiratory rate were detected with normal body temperature. No cyanotic mucus membranes were observed in any examined case.

### II-Gross findings:

Necropsy was performed on 4 calves. Cardiac anomalies were identified in all 4 cases as PFO (n=1), PDA (n=1) and VSD (n=2). VSD was located in the upper part of the membranous septum near the aortic root in 2 calves. VSD was accompanied by valvular hematoma in auriculo-ventricular valve in 1 calf, hydropericardium, right ventricular hypertrophy and dextroposition of the aorta in another calf (Figs. 1&2A). In two calves affected with VSD, necrotic bronchitis (1 calf) and atelectatic lungs (1 calf) were detected (Fig. 2B).

### III- Histopathological findings

Histopathological examination of different tissues from necropsied calves affected with CHD was described as the following:

-**Heart** in all cases revealed hyaline and hydropic degeneration, focal fibrosis in myocardium, edema and macrophages infiltration in pericardium in one VSD case. Valvular hematoma was a cyst filled with blood and lined by flattened cells surrounded by congested capillaries and mild edema (Fig. 3A-C).

- **Lung** lesions were seen in two cases with VSD. Diffuse atelectasis was observed in one of them characterized by collapsed alveoli with slit like

appearance, lined by cuboidal fetal epithelium (Fig. 3D). Microscopically, necrotizing bronchitis was seen characterized by necrosis and loss of bronchial epithelium, accumulation of necrotic caseated material and basophilic bacterial colonies in lumen of bronchi. Fibrinous exudate plugged lumen of surrounding alveoli (Fig. 4A).

-**Liver** showed few perivascular mononuclear cells (MNCs) infiltration with mild fibrous tissue proliferation in all cases. MNCs with eosinophils infiltration were seen in portal areas indicating extramedullary hematopoiesis. Hydropic degeneration in hepatocytes, activation of von kupffer cells and dilated sinusoids with RBCs and brown hemosiderin pigment were observed in two cases of VSD (Fig. 5 A&B).

-**Kidneys** in all cases showed chronic interstitial nephritis characterized by multifocal interstitial MNCs aggregation and fibrosis. Tubular casts, perivascular fibrosis, brown lipofuscin pigment was seen inside renal epithelium (Fig. 5C&D).

-**Spleen** in all cases showed hemorrhage in red pulp and perifollicular granulocytosis. Hemosiderosis in red pulp was found in one case of VSD (Fig. 6A-C).

-**Tonsils** showed crypt abscess with foci of dystrophic calcification and hypertrophy of lymphoid follicles were seen in all cases (Fig. 6D).

-**Lymph nodes** were normal in 3 cases and showed lymphoid hypertrophy in one VSD case.

- **Pancreas** showed remnant of primary islets in case of PDA and interstitial fibrosis in other cases (Fig. 7A&B).

-**Thyroid gland** showed nodular lymphocytic aggregation in one case of VSD (Fig. 7C).

-**Thymus** showed extramedullary hematopoiesis in all cases (Fig. 7D).

-**Compound stomach** showed only keratin abscesses in one case of VSD.

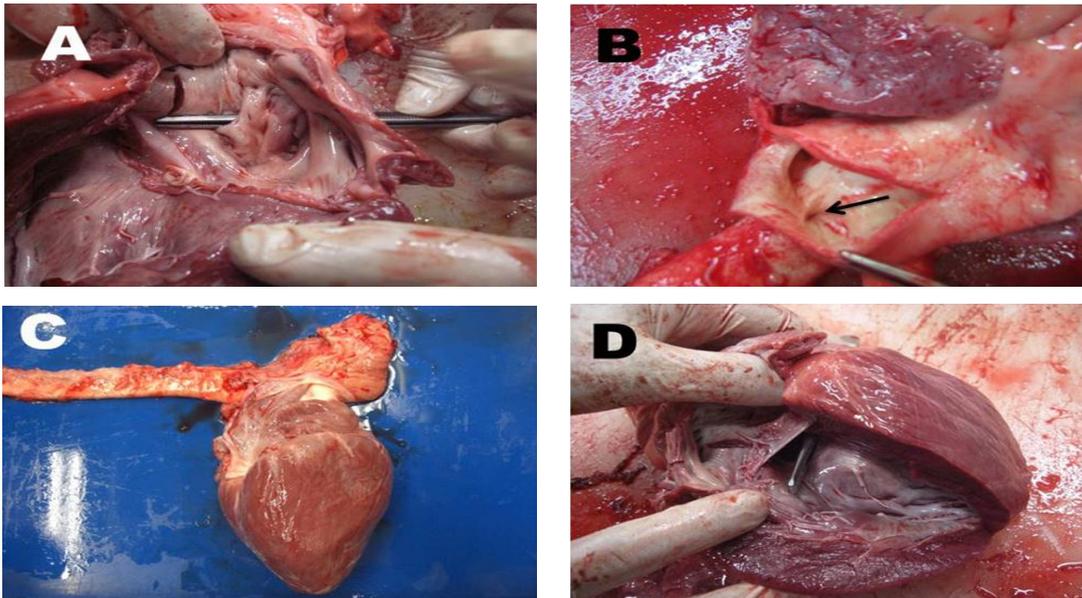
-**Intestinal tract** showed normal histological picture in 3 cases and in one case of VSD showed few eosinophils infiltration in mucosa.

### IV-Bacteriological findings

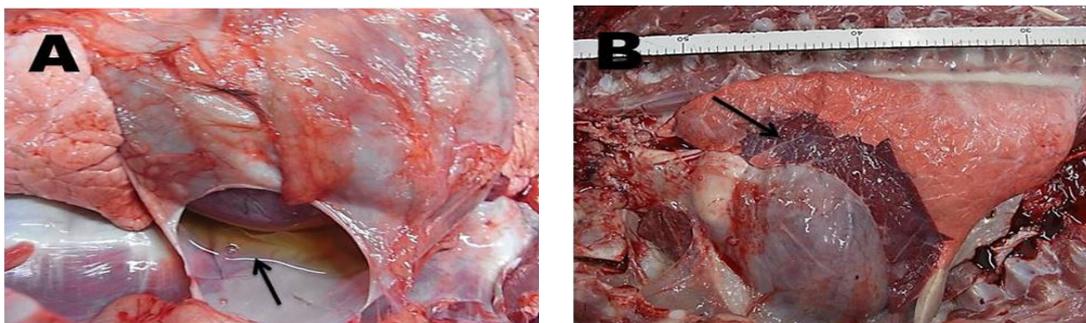
In one case, *P. multocida* was bacteriologically isolated from necrotic bronchitis lesion. On blood agar plate colonies appeared as small mucoid or dry colonies (1-2 mm in diameter). No hemolysis was detected macroscopically. *P. multocida* appeared as gram-negative, non-spore-forming coccobacilli with bipolar staining features.

### V-Immunohistochemical staining:

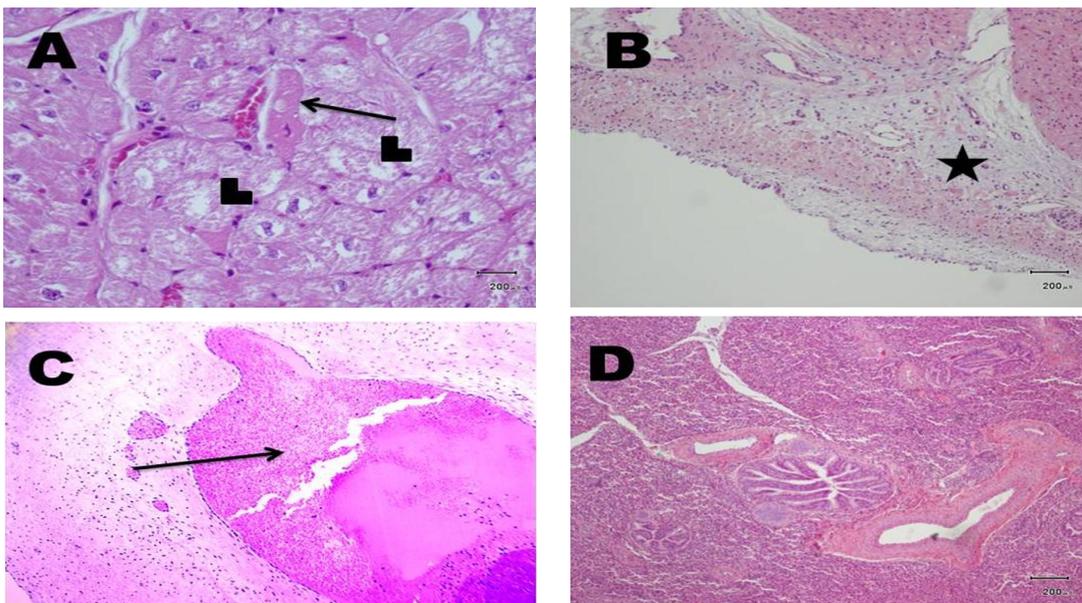
Positive brown immunolabelling for *P. multocida* was detected inside necrotic caseated material (Fig. 4B&C).



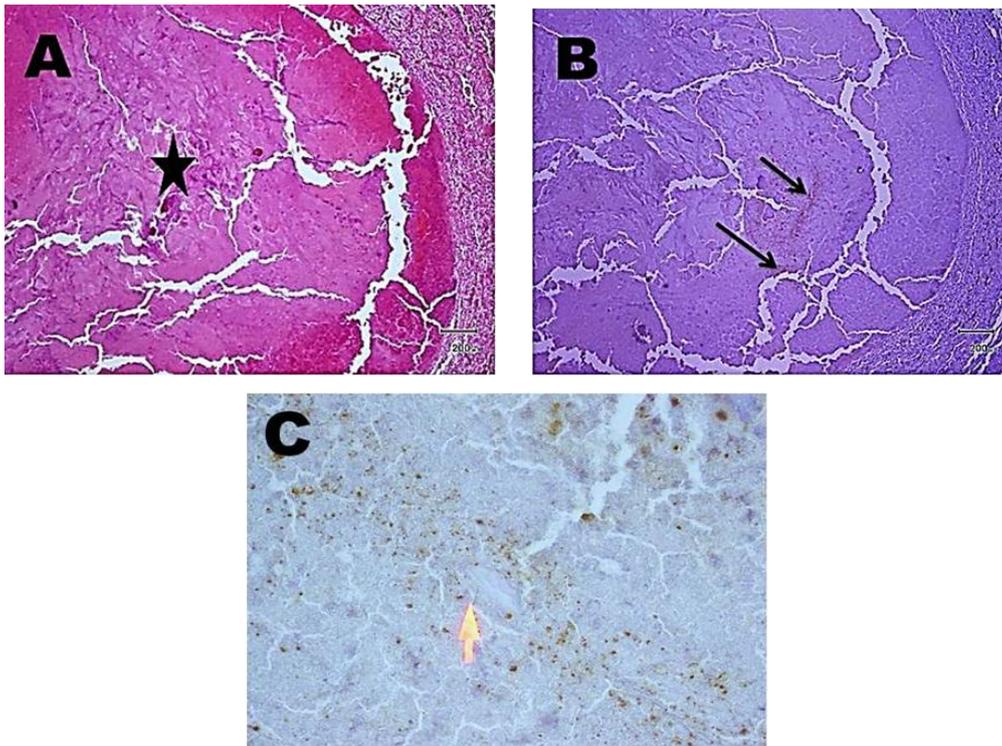
**Fig. 1 (A-D):** Hearts of necropsied calves. Gross pictures show: (A) PFO as an opening between both atria, (B) PDA as an opening of ductus arteriosus inside pulmonary artery (arrow), (C) dextroposition of the aorta and (D) VSD as an opening in the upper part of the membranous septum near the aortic root.



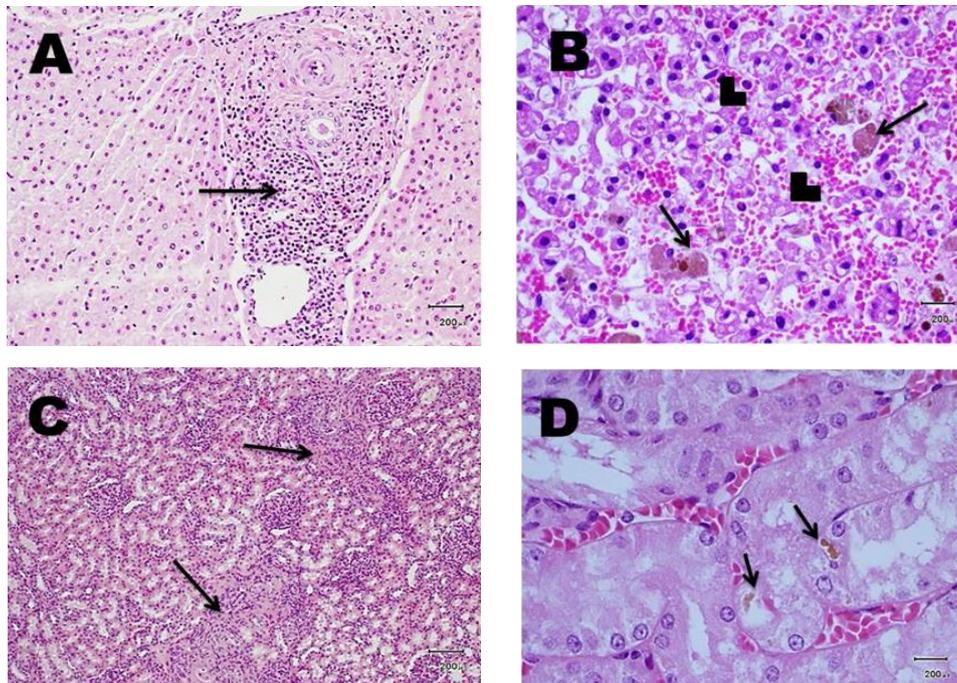
**Fig.2: (A):** Heart of a necropsied calf. Gross picture shows hydropericardium (arrow). **(B):** Lung of a necropsied calf. Gross picture shows atelectatic cranioventral portion of diaphragmatic lobe (arrow).



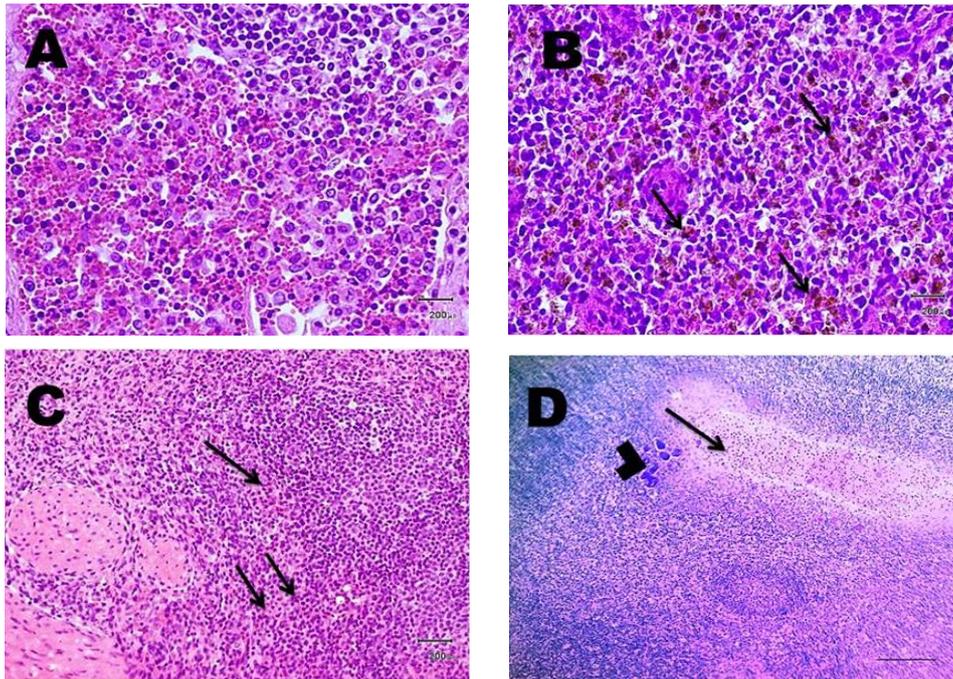
**Fig. 3:** Heart of a necropsied calf shows (A): hyaline degeneration (arrow) and hydropic degeneration (arrowheads) and (B): edema and leukocytic infiltration in pericardium. (C): Auriculo-ventricular valve shows hematoma lined by flattened cells (arrow) surrounded by congested capillaries. (D): Lung shows diffuse atelectasis characterized by collapsed alveoli with slit like appearance (H&E).



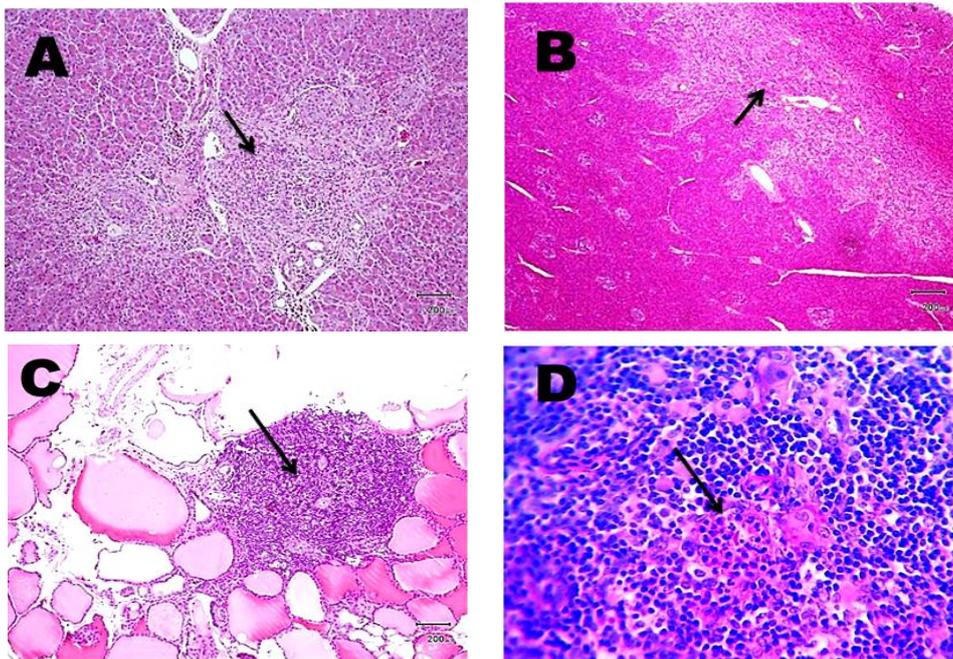
**Fig. 4(A-C):** Microscopic picture of Lung shows (A): necrotizing bronchitis characterized by accumulation of caseated necrotic material and basophilic bacterial colonies in lumen of bronchus (asterisk) H&E. Positive brown immunolabelling for *P. multocida* inside necrotic material is seen as indicated by (black arrows) in (B) and (yellow arrow) in (C) (IHC counterstained with Mayer's hematoxylin)



**Fig. 5:** Liver of necropsied calf shows (A) MNCs with eosinophils infiltration and mild fibrous tissue proliferation in portal area (arrow), (B) vacuolar degeneration in hepatocytes (arrowheads), dilated sinusoids with RBCs and brown hemosiderin pigment (arrows). Kidney of necropsied calf shows (C) chronic interstitial nephritis characterized by multifocal interstitial MNCs aggregation and fibrosis (arrows) and (D) brown lipofuscin pigment inside renal epithelium (arrows) H&E.



**Fig. 6:** Spleen of necropsied calf shows (A) hemorrhage, (B) hemosiderosis in red pulp and (C) perifollicular granulocytosis. Tonsils of necropsied calf show (D) crypt abscess (arrow) with foci of dystrophic calcification and (arrowhead). H&E



**Fig. 7:** (A&B) Pancreas shows (A) interstitial fibrosis and (B) remnant of primary islets. (C) Thyroid gland: shows nodular lymphocytic aggregation (arrow). (D) Thymus shows extra medullary hematopoiesis (arrow) H&E.

## DISCUSSION

In this study we reported CHD in 4 necropsied calves. Clinical signs of CHD were similar to those reported by Jafari Dehkordi and Hoseini (2016). All septal defects were high in location and ranged from 5 to 35 mm in diameter. VSD was previously described in 2 calves with focal fibrosis of the ventricular

endocardium, mitral, and aortic valves without infection (Buczinski *et al.*, 2006). VSD is most frequently located in the membranous part of the interventricular septum, adjacent to the aortic valve (Radostits *et al.*, 2000; Reef and McGuirk, 2002), and it causes free shunting of blood between the ventricles. Blood from the left ventricle is shunted towards the right ventricle and pulmonary arteries.

The excessive volume-flow overloads the pulmonary vascular tree and can lead to pulmonary hypertension (McGuirk *et al.*, 1984; Bonagura and Darke 1995). Since shunting occurs from left to right, the left heart demonstrates morphological changes typical of volume-flow overload (left atrial and left ventricle enlargement). When the pulmonary pressure exceeds the systemic pressure, reversal of the shunt may occur. That phenomenon was called the Eisenmenger's complex. Calves with a VSD usually had clinical signs of heart failure (Cordy and Ribelin, 1950; Belling 1961 and 1962; Sandusky and Smith 1981; West, 1988; Gavaghan *et al.*, 2001, Schwarzwald *et al.*, 2003). In some situations, the condition may occur without any clinical signs other than a cardiac murmur (Pipers *et al.*, 1985; Penrith *et al.*, 1994). VSD can damage the endocardium by blood flow turbulences and predispose to bacterial endocarditis (Brickner and Hillis 2000; Buczinski *et al.*, 2006). This complication of VSD has been reported in a goat (Parry *et al.*, 1982) and in a Holstein heifer (Besser and Knowlen, 1992). In our findings, pulmonary infection was detected instead. In PDA there was a significant left-to-right shunt which generated a large left ventricular volume overload. When the response of left ventricle failed dilation (primarily) and hypertrophy developed. These processes resulted in pulmonary hypertension and congestion. The right ventricle can be affected by the pulmonary pressure load and right ventricular hypertrophy can also develop. If the pulmonary resistance equals or exceeds the systemic vascular resistance, a right-to-left shunt happens (Radostitis *et al.*, 2007; Smith 2009). Here we described histopathology of tissues in CHD affected calves. In this report, extramedullary hematopoiesis and chronic interstitial nephritis were constant findings. Chronic kidney disease was reported in children with CHD (Morgan *et al.*, 2015). Chronic hypoxia was suggested to be the cause of both lesions. In cyanotic congenital heart disease, chronic hypoxia stimulates erythrocytosis through the stimulation of erythropoietin resulting in the increase of blood viscosity (DeFilippis *et al.*, 2007; Cordina and Celermajer 2010; Pichon *et al.*, 2012). Hyperviscosity influenced renal hemodynamics resulting in increased glomerular filtration (Nashat and Portal, 1967). The increased filtration fraction led to chronic nephropathological changes including glomerulosclerosis (Shankland *et al.*, 1994; Blantz and Gabbai, 1989). In addition, chronic hypoxia for 28 days in rats induced tissue changes including abnormal renal tubular structure and interstitial fibrosis (Truong *et al.*, 1992).

## CONCLUSION

Extramedullary hematopoiesis in liver and lymphoid organs and chronic interstitial nephritis were constant findings in CHD affected calves.

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### دراسات مرجعية على عجول الابقار المصابة بعيوب القلب الخلقي

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تم تشخيص عيوب قلب خلقية في ٤ عجول تم نشرها بعدما وردت الى المستشفى التعليمي بشها التابع لكلية الطب البيطري، جامعة المنصورة في الفترة من ٢٠١٤-٢٠١٦. عانت هذه العجول من ثقب اوفال المفتوح (عدد ١)، عيب علوى فى الحاجز البطينى (عدد ٢)، استمرارية القناة الشريانية (عدد ١). وقد صاحب العيب العلوى فى الحاجز البطينى وجود كيس دموي صمامى فى عجل واحد، تضخم عضلة القلب و انحراف الشريان الاورطى لليمين فى العجل الثانى. تم عزل الباستيريل مالوتوسيدا من اصابة رئوية وجدت مصاحبة للعيب العلوى فى الحاجز البطينى فى عجل واحد. كما تم فحص معظم انسجة العجول التى تم عمل الصفة التشريحية لها هيستوباثولوجيا. فوجد ان تخليق الدم خارج النخاع بالكبد والانسجة الليمفاوية مع التهاب الكلى البينى المزمن تغيرات مرضية ثابتة فيها جميعا.